

Research Article
(Araştırma Makalesi)

Ege Üniv. Ziraat Fak. Derg., 2022, 59 (4):601-609
<https://doi.org/10.20289/zfdergi.1195901>

Kağan TELLİ¹ 

Sibel SOYCAN ÖNEÇ^{1*} 

¹ Tekirdağ Namık Kemal University,
Agricultural Faculty, Department of Animal
Science, 59030, Süleymanpaşa, Tekirdağ,
Türkiye

* Corresponding author (Sorumlu yazar):
ssonenc@nku.edu.tr

Effects of sour yogurt addition to corn silage on silage fermentation, aerobic stability, and *in vitro* digestibility

Mısır silajına ekşi yoğurt ilavesinin silaj fermantasyonu, aerobik stabilite ve *in vitro* sindirilebilirlik üzerine etkisi

* This article has been summarized from the first author's master thesis. This article was supported by the Unit of Scientific Research Projects, Tekirdağ Namık Kemal University as project number NKUBAP.03.YL.21.305.

Received (Alınış): 30.10.2022

Accepted (Kabul Tarihi): 26.11.2022

ABSTRACT

Objective: The objective of this study was to determine the effects of sour yogurt addition on fermentation, aerobic stability, and *in vitro* digestibility of corn silages.

Materials and Methods: The research material consisted of harvested corn in October and sour yogurt. The experimental groups included the control without yogurt added, 10 g sour yogurt (Y10), 20 g sour yogurt (Y20), 30 g sour yogurt (Y30), 40 g sour yogurt (Y40), and 50 g sour yogurt. The sour yogurt was added to 1 kg of fresh corn.

Results: It was found that the amount of crude protein (CP) increased with the addition of yogurt ($p<0.01$). Water soluble carbohydrate (WSC) contents increased in the groups to which yogurt was added at a high level ($p>0.01$). In the study, the highest lactic acid (LA) content was determined as 69.44 and 69.48 g/kg dry matter (DM) in the Y40 and Y50 groups, while the lowest was determined as 64.64 g/kg DM in the control group ($p<0.01$). As the amount of yogurt added to corn increased (except Y50), the amount of ammonia nitrogen decreased ($p<0.01$). The addition of sour yogurt increased the amount of enzyme-soluble organic matter, *in vitro* organic matter digestibility, and metabolic energy (ME) contents of corn silages ($p<0.01$).

Conclusion: From the study conducted, it was concluded that the addition of sour yogurt to corn at the level of 20 and 30 g/kg had a positive effect on the chemical and microbiological properties of silages.

ÖZ

Amaç: Bu araştırma, ekşi yoğurt ilavesinin mısır silajlarının fermantasyonu, aerobik stabilitesi ve *in vitro* sindirilebilirlik üzerine etkilerini belirlemek amacıyla planlanmıştır.

Materyal ve Metot: Araştırma materyalini ekim ayında biçilmiş mısır ve ekşi yoğurt oluşturmuştur. Deneme gruplarını; yoğurt ilave edilmeyen kontrol, 10 g ekşi yoğurt (Y10), 20 g ekşi yoğurt (Y20), 30 g ekşi yoğurt (Y30), 40 g ekşi yoğurt (Y40) ve 50 g ekşi yoğurt (Y50) oluşturmuştur. Ekşi yoğurt 1 kg taze mısıra ilave edilmiştir.

Araştırma Bulguları: Yapılan çalışmada, yoğurt ilavesiyle ham protein miktarında artma olduğu bulunmuştur ($p<0.01$). Suda çözülebilir karbonhidrat içerikleri ise yoğurdun yüksek düzeyde ilave edildiği gruplarda artmıştır ($p>0.01$). Araştırmada, en yüksek laktik asit içeriği Y40 ve Y50 gruplarında, 69,44 ve 69,48 g/kg kuru madde olarak belirlenirken en düşük ise kontrol grubunda 64,64 g/kg kuru madde olarak belirlenmiştir ($p<0.01$). Mısıra ilave edilen yoğurt oranı arttıkça (Y50 hariç) amonyak azotu miktarı düşmüştür ($p<0.01$). Ekşi yoğurt ilavesi, mısır silajlarının enzimde çözünen organik madde miktarı, *in vitro* organik madde sindirilebilirliği ve metabolik enerji içeriklerini arttırmıştır ($P<0.01$).

Sonuç: Sonuç olarak, ekşi yoğurdun 20 ve 30 g/kg düzeyinde mısıra ilavesi, silajların kimyasal ve mikrobiyolojik özelliklerini olumlu yönde etkilemiştir.

Keywords: Corn, fermentation, silage, sour yogurt

Anahtar sözcükler: Mısır, fermantasyon kalitesi, silaj, ekşi yoğurt

INTRODUCTION

Corn is the most commonly used forage crop in silage production in our country because the WSC content is high, the buffer capacity is low, and the DM content is proportionally high. The quality of the silages obtained by harvesting the corn during the dough stage, which contains 28-42% DM, is high. However, if it is harvested early or late, the fermentation efficiency decreases (Filya, 2001a). It becomes necessary to add additives to the silage material with low fermentation efficiency.

Many chemical and biological additives have been developed for use in silage fermentation. Some additives positively affect silage fermentation, while others stabilize it aerobically. Apart from these, some additives increase the evaluation level of silages by animals by showing a probiotic effect (Kurtoğlu, 2011). In recent years, lactic acid bacteria (LAB) inoculants have been used more widely than chemical additives due to their effectiveness in guaranteeing silage fermentation and their biological origin. During silage fermentation, homofermentative LAB dominance ensures the effective use of WSC in the silage material. It increases the possibility of producing well-fermented silage even when the amount of WSC in the material is critical. However, due to its low effect on aerobic stability, the use of heterofermentative LAB has also started to become widespread (Filya, 2001b).

Yogurt is a fermented dairy product that contains live yogurt cultures formed by the lactic acid fermentation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria (Anonymous, 2005; Anonymous, 2006). A study determined that the DM amount of yogurts obtained with yogurt bacteria isolated from yogurts in Iskenderun varied between 14.50-17.64%, protein amount varied between 3.56-4.39%, and fat amount varied between 2.40-3.35%. In addition, the number of *S. thermophilus* was 8.79-9.32 log cfu/ml, and the number of *L. bulgaricus* was 8.3-9.38 log cfu/ml at the beginning of storage. It is reported that on the 21st day of storage, the number of *S. Thermophilus* varies between 8.28-9.09 log cfu/g, and the number of *L. bulgaricus* varies between 7.06-8.51 log cfu/g (Çelik, 2007). It has been reported that the rate of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* is high in the microbial population of sour yogurt (Tharmaraj & Shah, 2003).

Yogurt is a functional food that is loved and consumed in Türkiye. For this reason, sour yogurts are very common in homes, farms, and markets. In Tekirdağ, it was determined that yogurts that turn sour before their shelf life expires due to their sour taste are separated to be thrown away after about three months of observation. In today's world, where recycling is at the forefront, it is necessary to create alternative uses for yogurts whose shelf life has expired due to their sour taste. Studies have reported that sour yogurts contain active homofermentative LAB (Çelik, 2007). This situation led to the idea that sour yogurts have the potential to be used as a natural alternative to bacterial inoculants, which are commonly used in silage production, and that scientific data can be created by researching the subject.

A limited number of studies on the subject (Kiani et al., 2012) were found in the literature search. Kiani et al. (2012) examined the fermentation course of corn by adding 5% sour yogurt on a dry matter basis. It was reported that sour yogurt significantly decreased corn silage's pH, ash content, and ammonia nitrogen (NH₃-N) values, while Flieg score and crude protein increased. Researchers also stated that the effects of sour yogurt addition on corn silage quality should be investigated together with detailed chemical properties and animal production.

The objective of this study was to determine the effects of adding sour yogurt as a natural source of LAB to corn harvested in October on silage fermentation quality, aerobic stability, and *in vitro* digestibility.

MATERIAL and METHODS

Experimental design and ensiling process

The second product corn (Pioneer 32K61) was cut in the dough stage in October and chopped to 1.5-2 cm. Since the taste of yogurt is sour, it has been paid attention that consumers do not prefer it, and its expiration date is approaching. The fresh corn (FC) contained 23.70% DM, 6.2% CA of DM, 7.8% CP of DM, 117.9 g/kg DM of WSC, 280.1 mEq NaOH kg/DM of buffer capacity (Bc), 2.07 log cfu/g of

Lactobacilli, 2.0 log cfu/g of *Lactococci*, 1.91 log cfu/g of yeast, and 1.93 cfu /g of enterobacter and no mold, with a pH of 5.6. Sour yogurt (natural) had 12.99% DM, 0.97% CA, 5.48% CP, 90.29 g/kg DM of WSC, 115.95 g/kg DM of LA, 3.5 of pH, 4.54 log cfu/g of *Lactobacilli*, 2.0 log cfu/g of *Lactococci*, and 5.0 log cfu/g of yeast, with no mold before ensiling. Experimental groups included the control without yogurt added, 10 g sour yogurt (Y10), 20 g sour yogurt (Y20), 30 g sour yogurt (Y30), 40 g sour yogurt (Y40), and 50 g sour yogurt. The additives were added to 1 kg of fresh corn. The silage was made in a laboratory-scale fermentation system: Approximately 500 g of corn was weighed, and after the corn was placed in oxygen barrier polyethylene bags, it was vacuumed (CAS CVP-260PD) for 25 seconds at a vacuum level of 0.1 mPa (Tan & Büyüktosun, 2016). A total of 24 packages, 4 for each group, were left to ferment for 60 days under laboratory conditions (10-20 °C).

Physical and chemical analysis

Three different observers scored the silages on the day they were opened (60th day) in terms of color, odor, and structure (Deutsche Landwirtschafts Gesellschaft: DLG, Kılıç, 1986). Evaluation according to DLG, 16-20: excellent; 10-15: moderate; 5-9: medium; 0-4: poore. The pH of the silages was determined with a digital pH meter, the buffer capacity according to Playne & McDonald (1966), and LA by the spectrophotometric method (Barker & Summerson, 1941). Silage volatile fatty acids (acetic, butyric, propionic acid) and ethanol were determined by gas chromatography (Agilent Technologies 6890N gas chromatography, Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 um df. Max. Temp: 260°C. Cat. 11023) RL volatile fatty acids and ethanol (Wiedmeier et al., 1987). Ammonia nitrogen and WSC contents were determined according to Anonymous (1986). A neight-day aerobic stability test was carried out on samples developed by Ashbell et al. (1991). Flieg score was calculated from the dry matter and pH values of silages according to the formula below (Kılıç, 1986).

$$\text{Flieg score} = 220 + (2 \times \% \text{ DM} - 15) - 40 \times \text{pH}$$

According to this index, silage was considered “poor” when it had a score of <20; to be “low” with a score between 21 and 40; to be “medium” with a score between 41 and 60; to be “good” quality with a score between 61 and 80; and to be “excellent” when it had a score between 81 and 100 (Kılıç, 1986).

Microbial populations

Total mesophilic aerobic bacteria (TMAB), *Lactobacilli*, *Lactococci*, yeast, and mold analyzes were determined by the method developed by Seale et al. (1986). MRS agar (de Man Rogosa & Sharpe agar, Merck, Darmstadt, Germany) was used to detect *lactobacilli*. *Lactococci* were determined on M17 agar (Merck) (Meeske et al., 2002). In the enumeration of the yeast, malt extract agar and for enterobacter, violet red bile agar were used. The plates were incubated for three days at 30°C. The TMAB, *Lactobacilli*, *Lactococci*, mold, and yeast numbers of the silages were converted into logarithmic colony form units (cfu/g).

Nutrient analysis and *in vitro* digestibility

Dry matter, crude ash (CA) and organic matter (OM) contents of starting corn and silages were determined according to the weende analysis method (Karabulut & Canbolat, 2005). The crude protein (CP) content of feed samples was determined according to the methods of AOAC (1990). Neutral detergent fiber (NDF), acid detergent insoluble fiber (ADF), and acid detergent insoluble lignin (ADL) contents were determined according to the methods reported by Van Soest et al. (1991). Pepsin-cellulase digestibility was determined according to a modification method of De Boever et al. (1986). In the technique (VDLUFA,1997), pre-treatment with the pepsin-hydrochloric acid solution followed an incubation in water at 80°C for 45 minutes before the treatment by cellulase (Onozuka R-10 from *Trichoderma viride*, Merck). The solubility of the organic matter in cellulase (ELOS), the cellulase digestibility of the organic matter (DOM), and the insoluble organic matter in cellulase (EULOS) were derived as follows:

$$\text{ELOS (\%)} = \text{DM} - \text{CA} - \text{G}$$

G (%) = Loss upon ashing

$$\text{DOM (\%)} = (\text{ELOS} \times 10^2 / 100 - \text{CA \%})$$

$$\text{EULOS (g/kg)} = 1000 - \text{CA(g/kg DM)} - (\text{ELOS \%} \times 10)$$

The following equation reported by Weissbach et al. (1996) was used to determine the ME contents.

$$\text{ME (MJ/kg DM)} = 13.98 - 0.0147 \times \text{CA} - 0.0102 \times \text{EULOS} - 0.00000254 \times \text{EULOS}^2 + 0.00234 \times \text{RP}$$

CA, EULOS, RP with g/kg DM.

Statistical analyses

The statistical analyses were performed using SPSS software v.18 suite (SPSS, 2009). The effects of different treatments were evaluated using a one-way analysis of variance with Duncan's multiple-range tests. The statistically significant difference was chosen to be $p < 0.01$ (Efe et al., 2000).

RESULTS and DISCUSSION

The physical evaluation results of the silages opened on the 60th day of ensiling are tabulated in Table 1. According to the evaluation, the addition of yogurt to the corn silage had a positive effect on the odor. It did not show any negative effect on the structure and affected the color positively. Especially in the Y20 and Y30 groups, the smell is more pleasant, the color is more vivid and green, and the structure of the leaf-grain integrity has remained as fresh as the first day. When the Flieg scores of this study were compared with Kiani et al. (2012)'s study, it was determined that the higher scores were obtained. The control group's flieg point was higher than the results of Kavut & Soya (2012).

Table 1. Physical evaluation of silages and Flieg scores (n=4)

Çizelge 1. Silajların fiziksel değerlendirilmesi ve Flieg puanları (n=4)

Item	CON	SY10	SY20	SY30	SY40	SY50	SEM	p-Value
Smell	8.0	14.0	14.0	14.0	14.0	14.0	-	-
Structure	4.0	4.0	4.0	4.0	4.0	4.0	-	-
Colours	2.0	2.0	2.0	2.0	2.0	2.0	-	-
DLG point	14	20	20	20	20	20	-	-
Quality	Moderate	Excellent	Excellent	Excellent	Excellent	Excellent	-	-
Flieg score	116.12	117.48	122.49	121.32	118.31	118.21	0.84	0.213
Quality	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	-	-

CON: Control no additives, SY10:10 g sour yogurt, SY20:20g sour yogurt, SY30:30 g sour yogurt, SY40:40 g sour yogurt, SY50:50 g sour yogurt. SEM: Standard error of mean. ^{a-d}: Means with different letters in the same line are statistically significant ($p < 0.01$). According to Flieg score, silage was considered "poor" when it had a score of <20; to be "low" with a score between 21 and 40; to be "medium" with a score between 41 and 60; to be "good" quality with a score between 61 and 80; and to be "excellent" when it had a score between 81 and 100.

When the nutrients and cell wall contents of the corn silages were examined (Table 2), it was found that the amount of OM decreased in the experimental groups compared to the control ($p < 0.01$), while the crude ash contents increased. It was determined that the CA content increased with increasing the level of yogurt added to corn. This is because yogurt is rich in mineral substances. However, Kiani et al. (2012) determined a decrease in CA content in the yogurt-added group. This may be due to the usage rate.

Table 2. Chemical compositions of the corn silages (% in DM)**Çizelge 2.** Mısır silajlarının kimyasal kompozisyonu (% KM)

Item	CON	SY10	SY20	SY30	SY40	SY50	SEM	p-Value
OM	93.93 ^a	93.05 ^c	93.66 ^b	93.59 ^b	93.54 ^b	93.43 ^b	0.07	<0.001
CP	6.67 ^d	6.70 ^d	6.85 ^{cd}	7.09 ^c	7.57 ^b	7.88 ^a	0.11	<0.001
CA	6.07 ^c	6.95 ^a	6.34 ^b	6.41 ^b	6.46 ^b	6.57 ^b	0.07	<0.001
NDF	59.20 ^a	58.38 ^b	57.43 ^c	54.85 ^d	50.90 ^e	51.11 ^e	0.81	<0.001
ADF	28.86 ^a	28.26 ^b	27.61 ^c	25.84 ^d	25.23 ^e	25.05 ^e	0.37	<0.001
ADL	2.97 ^a	2.53 ^b	2.24 ^b	2.15 ^{bc}	1.71 ^d	1.76 ^{cd}	0.11	<0.001
Hemicellulose	30.34 ^d	30.11 ^a	29.82 ^a	29.02 ^b	25.67 ^c	26.05 ^c	0.47	<0.001
Cellulose	25.90 ^a	25.73 ^{ab}	25.37 ^b	23.69 ^c	23.52 ^c	23.29 ^c	0.27	<0.001

CON: Control no additives, SY10: 10 g sour yogurt, SY20: 20g sour yogurt, SY30: 30 g sour yogurt, SY40: 40 g sour yogurt, SY50: 50 g sour yogurt, OM: Organic matter, CP: Crude protein, CA: Crude ash, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin. SEM: Standard error of mean. ^{a-e}: Means with different letters in the same line are statistically significant ($p < 0.01$).

In the study, CP increased with the addition of yogurt ($p < 0.01$). This was due to the high CP content of yogurt. Kiani et al. (2012) reported that the addition of 5% sour yogurt caused an increase in the amount of CP and a decrease in $\text{NH}_3\text{-N}$ in corn silages. In the study, sour yogurt increased CP amounts similar to that of Kiani et al. (2012). Despite the increased amount of yogurt in corn silages, $\text{NH}_3\text{-N}$ decreased similarly to that of Kiani et al. (2012). The increase in CP and decrease in $\text{NH}_3\text{-N}$ with the addition of sour yogurt to corn silages may be due to the yogurt's slowing of proteolysis despite the increasing proportion of yogurt. However, the low CP amounts of corn silages were also increased. Also, when large amounts of proteolysis occur in silages, additional protein sources are required even if the total CP of the ratio appears sufficient to achieve optimum milk production. Therefore, proteolysis in silage-making can significantly affect the cost of milk production (Muck, 1988). The NDF, ADF and ADL contents of the corn silages were highest in DM in the control group as 59.20%, 28.86%, 2.97%, respectively, while the lowest were Y40 (50.95%, 25.23%, 1.71%) and Y50 (51.11%, 25.05%, 1.76) groups ($p < 0.01$). Marbun et al. (2020) reported that different LAB inoculants did not significantly affect NDF and ADF in corn silages. There was a decrease in NDF, ADF, and ADL due to the absence of cell walls among the nutritional components of sour yogurt.

Table 3. Fermentation quality of corn silages**Çizelge 3.** Mısır silajlarının fermantasyon kalitesi

Item	CON	SY10	SY20	SY30	SY40	SY50	SEM	p-Value
DM,%	22.22 ^b	22.91 ^a	23.41 ^a	22.83 ^a	21.99 ^b	21.94 ^b	0.13	<0.001
pH	3.33	3.33	3.23	3.23	3.27	3.27	0.02	0.493
WSC, g/kg DM	11.23 ^c	10.26 ^{de}	10.03 ^e	10.44 ^d	11.89 ^b	13.36 ^a	0.28	<0.001
LA, g/kg DM	64.64 ^d	66.52 ^c	66.52 ^c	68.36 ^b	69.44 ^a	69.48 ^a	0.43	<0.001
AA, g/kg DM	31.84 ^a	31.13 ^a	28.50 ^b	27.54 ^c	25.67 ^d	23.63 ^e	0.70	<0.001
BA, g/kg DM	1.66 ^a	1.58 ^a	1.44 ^b	1.34 ^c	1.26 ^c	1.15 ^d	0.04	<0.001
PA, g/kg DM	2.12 ^e	2.27 ^d	2.36 ^c	2.44 ^b	2.59 ^a	2.57 ^a	0.04	<0.001
Ethanol, g/kg DM	2.10 ^d	2.22 ^c	2.36 ^b	2.49 ^a	2.55 ^a	2.50 ^a	0.04	<0.001
LA/AA	2.03 ^f	2.14 ^e	2.33 ^d	2.48 ^c	2.71 ^b	2.94 ^a	0.08	<0.001
$\text{NH}_3\text{-N}$, g/kg TN	127.98 ^a	122.03 ^b	114.96 ^c	111.77 ^d	112.57 ^d	116.22 ^c	1.39	<0.001
DM loss,%	1.64	1.37	1.33	1.36	1.44	1.46	0.06	0.814

CON: Control no additives, SY10: 10 g sour yogurt, SY20: 20g sour yogurt, SY30: 30 g sour yogurt, SY40: 40 g sour yogurt, SY50: 50 g sour yogurt, DM: Dry matter, WSC: Water-soluble carbohydrates, LA: Lactic acid, AA: Acetic acid, BA: Butyric acid, PA: Propionic acid, LA/AA: The ratio of lactic acid and acetic acid, $\text{NH}_3\text{-N}$: Ammonia nitrogen, TN: Total nitrogen, DM loss: Dry matter loss, SEM: Standard error of the mean. ^{a-f}: Means with different letters in the same line are statistically significant ($p < 0.01$).

The fermentation quality components of corn silages are given in Table 3. While the DM of fresh corn, which is the starting material of the research, was found to be 23.70%, it was found to be between

21.95%-23.41% at the end of the 60-day fermentation. It was found that sour yogurt increased DM when added at a low level, while DM was similar to control in Y40 and Y50 groups. This may be due to the 12.99% DM content of yogurt. Indeed, Kiani et al. (2012) reported that sour yogurt did not affect the DM contents of corn silage. In a study, it was reported that LAB inoculants did not have a significant effect on DM content (Sucu, 2009). The study determined that pH values were not affected by the addition of yogurt ($p>0.01$). Since corn is in the group of easily ensiled forages, it contains 117.9 g/kg DM WSC in the starting material. The WSC level of the starting material corn is sufficient for good fermentation development (Kurtoğlu, 2011), and it is expected no difference in pH between the groups.

The WSC contents increased in the groups to which yogurt was added at a high level (Table 3). In the study, the highest LA content was determined as 69.44 and 69.48 g/kg DM in the Y40 and Y50 groups, while the lowest was determined as 64.64 g/kg DM in the control group ($p<0.01$). In making ensiled forage, there must be LAB in order not to spoil the silage and sufficient WSC in order for them to produce lactic acid (Filya, 2000). In the study, the addition of yogurt showed an encouraging effect on the development of *Lactobacilli* numbers (Table 4); while the numbers of *Lactobacilli* increased, the yeast numbers in the control and yogurt groups also increased. It has been reported that carbohydrate sources in silage media activate the growth of some anaerobic bacteria, primarily lactic acid bacteria (Bolsen et al., 1996). The increase in *Lactobacilli* and yeast numbers was due to the addition of yogurt with a high WSC content. The WSC content of sour yogurt was determined as 90.29 g/kg DM, and the LA amount was determined as 115.95 g/kg DM. The highest ammonia nitrogen ($\text{NH}_3\text{-N}$) was 127.98 g/kg TN in the control group, while the lowest was 111.77 g/kg TN in the Y30 group ($p<0.01$). As the amount of yogurt added to corn increased, the amount of ammonia nitrogen decreased, but the increase in the level caused an increase in the losses in the form of $\text{NH}_3\text{-N}$ in direct proportion to the increase in the protein content of the Y50 group ($p<0.01$). This result was due to the high CP level of yogurt.

Table 4. Microbiological analysis results of corn silages, log₁₀ cfu/g

Çizelge 4. Mısır silajlarının mikrobiyolojik analiz sonuçları, log₁₀ cfu/g

Item	CON	SY10	SY20	SY30	SY40	SY50	SEM	p-Value
<i>Lactobacilli</i>	4.13 ^c	4.42 ^{bc}	5.63 ^a	5.80 ^a	4.61 ^{bc}	4.66 ^b	0.15	<0.001
<i>Lactococci</i>	2.34	2.46	2.78	2.70	2.67	2.76	0.05	0.019
TMAB	4.19	4.21	4.24	4.16	4.53	4.48	0.04	0.003
Yeast	4.27 ^{ab}	4.38 ^a	4.36 ^a	4.03 ^b	4.26 ^{ab}	4.26 ^{ab}	0.03	<0.001
Mould	ND	ND	ND	ND	ND	ND	-	-
Enterobacter	ND	ND	ND	ND	ND	ND	-	-

CON: Control no additives, SY10: 10 g sour yogurt, SY20: 20g sour yogurt, SY30: 30 g sour yogurt, SY40: 40 g sour yogurt, SY50: 50 g sour yogurt, TMAB: Total mesophilic aerobic bacteria, ND: Not detected, SEM: standard error of the mean. ^{a-c}: Means with different letters in the same line are statistically significant ($p<0.01$).

Microbiological analysis results are given in Table 4. The addition of sour yogurt increased *Lactobacilli* numbers of corn silages, did not affect *Lactococci*, TMAB numbers, and caused a significant ($p<0.01$) decrease in yeast numbers. Mold and enterobacter were not detected in all silages. In studies with homofermentative LAB inoculants, it has been reported that *Lactobacilli* numbers in corn silages generally increase (Muck, 1993; Filya, 2001), their effects on yeast and mold numbers vary and decrease (Filya, 2002a), do not affect (Filya, 2002a, b) or increase (Weinberg, et al., 1993; Kleinschmit et al., 2005). In the study, yeast numbers increased in Y10 and Y20 groups compared to the control, as in Weinberg et al. (1993) and Kleinschmit et al. (2005), but did not affect the Y30, Y40, and Y50 groups, as in Filya (2002a,b). The high yeast numbers in silages were due to the high yeast numbers of corn (1.91 cfu/g) and sour yogurt (5.0 cfu/g), which are the starting materials. Filya (2003) reported *Lactobacilli*, yeast, and mold numbers of fresh corn as 3.86, 4.06, and 2.58 cfu/g, respectively. Despite the low pH of the silages, the high yeast numbers can be explained by the fact that the yeasts are very resistant to extreme environmental conditions (Kurtoğlu, 2011).

The results of the aerobic stability test applied to the silages for eight days are presented in Table 5. The addition of yogurt decreased the amount of DM on the 4th and 8th days of aerobic stability compared to the control and positively affected the pH values ($p<0.01$). The TMAB numbers increased relative to the control group in the aerobic period, encouraging yeast numbers' development. Yeast numbers of the control and yogurt groups were found above the critical level (5 cfu/g) in the aerobic period. Mold growth did not occur on the 4th day of the aerobic period and anaerobic period. On the 4th day of the aerobic period, no significant difference could be determined between the groups in DM and pH, while on the 8th day, the pH value of the control group was lower than the groups with yogurt added. This can be explained by the fact that the amount of mold increased due to the addition of yogurt and prolonged contact with oxygen.

Table 5. Aerobic stability test results of corn silages

Çizelge 5. Mısır silajlarının aerobik stabilite test sonuçları

Item	CON	SY10	SY20	SY30	SY40	SY50	SEM	<i>p</i> -Value
4.day								
DM, %	21.05	18.97	20.62	20.17	19.90	19.31	0.26	0.159
pH	5.87	6.07	5.83	5.90	5.93	6.03	0.26	0.019
TMAB, cfu/g	6.33 ^{ab}	6.44 ^a	6.33 ^{ab}	6.26 ^{ab}	6.19 ^b	6.37 ^{ab}	0.02	0.003
Yeast, cfu/g	6.32 ^a	6.33 ^a	5.44 ^b	5.26 ^c	5.17 ^c	5.47 ^b	0.12	<0.001
Mould, cfu/g	ND	ND	ND	ND	ND	ND	-	-
8.day								
DM, %	19.97	17.92	19.99	19.62	18.51	18.71	0.35	0.437
pH	5.87 ^b	6.50 ^a	6.37 ^a	6.40 ^a	6.37 ^a	6.47 ^a	0.05	<0.001
TMAB, cfu/g	6.44	6.42	6.41	6.41	6.42	6.32	0.01	0.103
Yeast, cfu/g	6.47	6.41	6.46	6.42	6.36	6.46	0.01	0.03
Mould, cfu/g	2.65 ^a	2.20 ^a	ND	ND	2.53 ^a	2.67 ^a	0.29	<0.001

CON: Control no additives, SY10: 10 g sour yogurt, SY20: 20g sour yogurt, SY30: 30 g sour yogurt, SY40: 40 g sour yogurt, SY50: 50 g sour yogurt, DM: Dry matter, TMAB: Total mesophilic aerobic bacteria, ND: Not detected, SEM: standard error of the mean. ^{a-c}: Means with different letters in the same line are statistically significant ($p<0.01$).

The ELOS, EULOS, DOM, and ME contents of silages are given in Table 6. The addition of yogurt increased ELOS amounts and was found as 59.17%, 61.27%, 63.22%, 64.42%, 65.25 and 66.07%, respectively, in DM in control, Y10, Y20, Y30, Y40, and Y50 groups ($p<0.01$). The EULOS decreased with the increase of ELOS ($p<0.01$).

Table 6. *In vitro* digestibility and ME contents of corn silages (in DM).

Çizelge 6. Mısır silajlarının *in vitro* sindirilebilirlik ve ME içerikleri (KM'de).

Item	CON	SY10	SY20	SY30	SY40	SY50	SEM	<i>p</i> -Value
ELOS, %	59,17 ^f	61,27 ^e	63,22 ^d	64,42 ^c	65,25 ^b	66,07 ^a	0.58	<0.001
DOM	62,99 ^f	65,85 ^e	67,50 ^d	68,83 ^c	69,76 ^b	70,72 ^a	0.63	<0.001
EULOS	347,60 ^a	317,76 ^b	304,41 ^c	291,73 ^d	282,88 ^e	273,57 ^f	5.96	<0.001
ME _{EULOS}	2391,28 ^f	2421,26 ^e	2470,93 ^d	2496,32 ^c	2515,66 ^b	2532,96 ^a	12.27	<0.001

CON: Control no additives, SY10: 10 g sour yogurt, SY20: 20g sour yogurt, SY30: 30 g sour yogurt, SY40: 40 g sour yogurt, SY50: 50 g sour yogurt, ELOS: Solubility of the organic matter in cellulase; EULOS: Insoluble organic matter in cellulase DOM: The cellulose digestibility of organic matter, ME_{EULOS}: Metabolic energy predicted with EULOS, SEM: standard error of the mean. ^{a-f}: Means with different letters in the same line are statistically significant ($p<0.01$).

In vitro organic matter digestibility of corn silages was increased with the addition of yogurt ($p<0.01$). The addition of yogurt also caused an increase in the ME content of corn silages, and the highest ME was found to be 2532.96 kcal/kg DM in Y50 group. The addition of yogurt to corn during ensiling increased the ME contents ($p<0.01$). Meeske et al. (2002) reported that inoculants numerically increased the *in vitro* DOM content of corn silages. In the study, the addition of yogurt to corn silages

positively affected the feed value. This showed that the use of corn silages with added sour yogurt in ruminant rations ratios has the potential to improve animal productivity.

CONCLUSION

Sour yogurt, which was investigated to determine its potential as an alternative to commercial LAB inoculants, increased *Lactobacilli* numbers and efficacy by promoting LAB development. Accordingly, the conversion of sugars to lactic acid increased, and lactic acid, which was present at a high rate in the medium, decreased the pH. However, proteolysis was inhibited, and the degradation of proteins to ammonia was also reduced. In addition, the amount of organic matter dissolved in the enzyme increased, and the digestibility of organic matter and ME content increased in parallel.

In the study, the high yeast numbers were due to the high yeast numbers of corn and sour yogurt at the beginning. It is suggested that future research should be conducted in order to determine the effects of different silage plants by adding them by diluting at the level of 20 and 30 g/kg and different rates, taking into account the higher dry matter content.

ACKNOWLEDGMENTS

The authors would like to thank the Coordination of Scientific Research Projects of Tekirdağ Namık Kemal University for its financial support and Assoc. Prof. Dr. Önder CANBOLAT.

REFERENCES

- Anonymous, 2006. Türk Standartlar Enstitüsü TS 1330. Yoğurt Standardı. TSE, Ankara, 1-11.
- Anonymous, 1986. The Analysis of Agricultural Material. Third Ed. Reference Book, No: 427, London, 428 pp.
- Anonymous, 2005. Türk Gıda Kodeksi Yönetmeliği. T.C. Tarım ve Köyişleri Bakanlığı, ilk yayın 16.11.1997 tarih ve 23172 sayılı Resmî Gazete, son yapılan değişiklik 30.06.2005 tarih ve 25861 sayılı Resmi Gazete Ankara: 2005.
- AOAC, 1990. Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists: Arlington, WA, USA, 771 pp.
- Ashbell, G., Z. G. Weinberg, A. Azrieli, Y. Hen & B. Horev, 1991. A simple system to study the aerobic deterioration of silages. Canadian Agricultural Engineering, 33: 391-393.
- Barker, S.B. & W.H. Summerson, 1941. The colorimetric determination of lactic acid in biological material. Journal of Biological Chemistry, 138: 535-554.
- Çelik, E. S., 2007. Geleneksel Yoğurtlardan İzole Edilen Laktik Asit Bakterilerinin Aroma Bileşikleri ve Ekzopolisakkarit Üretimlerinin Belirlenmesi. İzmir Teknoloji Enstitüsü (Basılmamış) Yüksek Lisans Tezi, İzmir.
- De Boever, J.L., B.G. Cottyn, F.X. Buysse, F.W. Wainmann & J.M. Vanacker, 1986. The use of an enzymatic technique to predict digestibility, metabolizable and net energy of compound feedstuffs. Animal Feed Science Technology, 14: 203-214.
- Efe, E., Y. Bek & M. Şahin, 2000. Statistical Methods with Solutions in SPSS. Kahramanmaraş Sütçü İmam University Pub. Kahramanmaraş, Turkey, 209 pp.
- Filya, İ., 2001a. Silaj Teknolojisi. Hakan Ofset, İzmir. 55 s.
- Filya İ., 2001b. Silaj fermantasyonu. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 32 (1): 87-93.
- Filya, İ., 2002a. Laktik asit bakteri inokulantlarının mısır ve sorgum silajlarının fermantasyon, aerobik stabilite ve in situ rumen parçalanabilirlik özellikleri üzerine etkileri. Turkish Journal of Veterinary & Animal Science, 26 (4): 815-823.
- Filya, İ., 2002b. Laktik asit ve laktik asit+enzim karışımı silaj inokulantlarının mısır silajı üzerine etkileri. Turkish Journal of Veterinary & Animal Science, 26: 679-687.
- Filya, I., 2003. The Effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. Journal of Applied Microbiology, 95: 1080-1086.

- Kavut, Y.T. & H. Soya, 2012. Ege Bölgesi koşullarında bazı mısır (*Zea mays* L.) çeşitlerinin silaj kalite özellikleri üzerinde bir araştırma. Ege Üniversitesi Ziraat Fakültesi Dergisi, 49 (3): 223-227.
- Kiani, A., R. Fallah & A. Azarfar, 2012. Effect of adding sour yoghurt and dough as bacterial inoculant on quality of corn silage. African Journal of Biotechnology, 11(50):11092-11095.
- Kilic, A., 1986. Silo Feed (Instruction, Education and Application Proposals). Bilgehan Press, İzmir, Turkey, 327 s.
- Kleinschmit, D. H., R. J. Schmidt & L. J. Kung, 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. Journal of Dairy Science, 88: 2130-2139.
- Kurtoğlu, V., 2011. Silage and Silage Additives. Aybil Press, Konya-Turkey, 356 s.
- Marbun, T.D., K. Lee, J. Song, C.H. Kwon, D. Yoon, M.S. Lee, J. Kang, C. Lee, S. Cho & E.J. Kim, 2020. Effect of lactic acid bacteria on the nutritive value and *in vitro* ruminal digestibility of maize and rice straw silage. Applied Science, 10: 7801.
- Meeske, R., G. Van der Merwe, J. Greyling & C. Cruywagen, 2002. The effect of adding an enzyme containing lactic acid bacterial inoculant to big round bale oat silage on intake, milk production and milk composition of Jersey cows. Animal Feed Science & Technology, 97: 159-167.
- Karabulut, A. & Ö. Canbolat, 2005. Yem Değerlendirme ve Analiz Yöntemleri. Uludağ Üniversitesi Basımevi, Bursa, 520 s.
- Playne, M.J. & P. McDonald, 1966. The buffering constituent of herbage and silage. Journal of Science Food Agriculture, 17: 264-268.
- Seale, D.R., G. Pahlow, S.F. Spoelstra, S. Lindgren, F. Dellaglio & J.F. Lowe, 1990. "Methods for the microbiological analysis of silage, 147". Proceeding of The Eurobac Conference, (12-16 August, Uppsala, Sweden), 164 pp.
- SPSS, 2009. PASW Statistics for Windows, Version 18.0. Chicago, SPSS Inc.
- Sucu, E., 2009. Laktik Asit Bakteri İnokulantlarının Mısır Silajının Fermantasyon ve Aerobik Stabilité Özellikleri ile Rumen Ekolojisi Üzerine Etkileri. Uludağ Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış) Doktora Tezi, 134 s.
- Tan, F. & N. Büyüktosun, 2016. Effect on density and dry matter losses of vacuum in vacuum-packed polythene bags silage. International Journal of Current Research, 8 (06):33185-33188.
- Tharmaraj, N. & N.P. Shah, 2003. Selective enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, bifidobacteria, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and propioni bacteria. Journal of Dairy Science, 86: 2288-2296.
- VDLUFA, 1997. Methodenbuch Band III. Die Chemische Untersuchung von Futtermitteln. Verband Deutscher Landwirtschaftlicher Untersuchung und Forshung Anstalten (Hrsg.), VDLUFA-Verlag, Darmstadt, Germany, 190 pp.
- Weinberg, Z.G., G. Ashbell, Y. Hen & A. Azrieli, 1993. The effect of applying lactic acid bacteria ensiling on the aerobic stability of silages. Journal of Applied Bacteriology, 75: 512-518.
- Weißbach, F., S. Kuhla & L. Schmidt, 1996. "Schätzung der umsetzbaren Energie von Grundfutter mittels einer Cellulase-Methodei, 10-115". In: Giesecke, D. (Hrsg.) DLG, 115 pp.
- Wiedmeier, R.D., M.J. Arambel & J.L. Walters, 1987. Effect of orally administered pilocarpine on ruminal characteristics and nutrient digestibility in cattle. Journal of Dairy Science, 70: 284-289.